INTERIM REPORT **(Submitted in Lieu of Final Report for Contract No. 96-0257)**

Submitted to: California Department of Pesticide Regulation
September 30, 1998

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Project Title: Investigation of an augmentation program for grape and longtailed mealybugs and classical biological control of the obscure mealybug

Summary:

Classical biological control of the obscure mealybug was monitored in Central Coast vineyards, where releases of two imported parasitoids (*Pseudaphycus flavidulus* and *Leptomastix epona*) began in 1997. Both species overwintered successfully, and releases of the parasitoids continued. Monitoring continued in the North Coast's Carnerros region, where a potentially cold-tolerant strain of the mealybug destroyer beetle has been released since 1994 for control of the grape mealybug. Unlike previous seasons, none of the beetles were recovered in 1998, and there was a notable lack of parasitization observed in the area compared with parasitization rates in the Central Valley. At UCB, rearing methods were explored for two native parasitoids of the grape mealybug (*Acerophagus notativentris* and *Pseudaphycus angelicus*) to lay the groundwork for future augmentative control programs. Laboratory cultures were started for the longtailed mealybug, grape mealybug and the two native parasitoids. Another easily reared mealybug species, the striped mealybug, was tested and rejected as an alternate host for rearing either the native and imported parasitoids. Experiments confirmed that *P. angelicus* can parasitize the longtailed mealybug as well as the grape mealybug, and further experiments are underway to determine its potential as a biological control agent. Colonies of the two native parasitoids are now being multiplied for use in inoculative experiments tentatively planned for Spring, 1999. A small colony of grape mealybug has also been established on caged grapes in a greenhouse, for use in assessing parasitoid effectiveness. Efforts to start cultures of a native cecidomyid mealybug predator are beginning in Fall, '98.

Results and Discussion (by objective):

1.) Investigate the potential of rearing two parasitoids (*Acerophagus notativentris* and *Pseudaphycus angelicus*) and a cecidomyid midge for control of grape and longtailed mealybugs.

1a.) Rearing studies

A reliable supply of mealybugs is necessary for rearing parasitoids and for conducting many of the proposed experiments. The quality and quantity of the mealybugs available are largely dependent upon the choice of rearing media, which can also effect the quality of parasitoids. Since two of the mealybug species in question—the longtailed and grape mealybugs—are known

to be difficult to maintain in culture, our first priority was to find the best rearing medium available.

At the beginning of the study, cultures of the obscure mealybug, *Pseudococcus viburni* (Signoret) had already been started at UCB in order to maintain colonies of the mealybug's two imported parasitoids, *Pseudaphycus flavidulus* (Brethes) and *Leptomastix epona* (Walker). These parasitoids were imported from Chile in 1993 and 1997 by K. Daane. Cultures of citrus mealybug *Planococcus citri* (Risso) and the citrophilus mealybug *Pseudococcus calceolariae* (Maskell) were also in place for the rearing of two strains of *Cryptolaemus montrouzieri* Mulsant. One of these *Cryptolaemus* strains was imported by Prof. Ken Hagen, and is thought to be more cold-tolerant than those imported previously. Laboratory studies on this cold-tolerant strain continued.

A number of researchers and insectary managers were consulted regarding techniques for rearing the grape, longtailed and obscure mealybugs (see Attachment A). Four varieties of potatoes, three varieties of squash, and several plant species were then informally compared for their suitability in rearing five mealybug species. Three potato varieties have been tried so far in the mealybug cultures: organically grown "Norkotah" seed potatoes, grade "B" commercial russet potatoes, and organically grown "Red Lasota" potatoes. Based on observations of our mealybug cultures, any potato variety seems acceptable for the citrus and citrophilus mealybugs, even potatoes without large sprouts. For obscure, grape and longtailed mealybugs all three potato varieties were acceptable but crawlers were less likely to settle on the "Norkotah" variety, and the presence of large sprouts appeared more important for these mealybug species. Greening of the sprouts caused by exposure to light also appeared to inhibit mealybug development. Evaluation of the "Red Lasota" variety (which began in August) suggests that this is the preferred variety for mealybug settling, although it may be quicker to rot than the thicker-skinned russet varieties.

The UCB lab also experimented with three squash varieties for mealybug rearing: acorn, butternut, and kabocha ("Japanese pumpkin"). Acorn squash was tried only with obscure mealybug, and there was little or no settling of mealybugs observed. Butternut was tried with both obscure and grape mealybugs, and both species were successfully reared to maturity. The success with grape mealybug cannot be fully evaluated until second generation crawlers appear. Kabocha has been tried with longtailed and with grape mealybugs only, and appears acceptable only for the grape mealybug. On all three varieties, the surface of the squash was scored lightly to facilitate mealybug feeding.

In addition, grape mealybug cultures have been started on ice plant (*Mesembryanthemum* sp.) and sea fig (*Carpobrotus edulis*). Results from these trials will be presented in the final report.

Longtailed mealybug cultures were reared on pothos ivy (*Epipremnum aureum*), Dracaena (*Cordyline terminalis*), and sprouting russet potatoes. Pothos ivy appeared less suitable due to the waxiness of its leaves, which often caused mealybugs to fall off, and the pothos culture was therefore discontinued. After an initial explosion in crawler production, all the longtailed

mealybug colonies have declined somewhat. The causes are still being evaluated. A preliminary evaluation of the rearing media tried to date is presented in Attachment B.

An as-yet-unidentified cecidomyid midge has been observed feeding on grape mealybug eggs in the Central Valley on several occasions. Judging by prior successes with cecidomyid aphid predators, the species may hold potential as an augmentative control agent. We plan to begin culturing the midge this Fall.

1b.) Test the longtailed mealybug as an insectary host for both parasitoid species

Longtailed mealybugs were exposed to both *P. angelicus* and *A. notativentris* parasitoids. No *A. notativentris* have yet been recovered from longtailed mealybug, but *P. angelicus* reproduced readily on the species. It remains unclear whether the longtailed mealybug would actually prove easier to rear than grape mealybug. Because it does not produce an ovisac, propagating a culture is somewhat more laborious, and the mealybug does not appear to reproduce rapidly in any case.

Colonies of another mealybug species, the striped mealybug *Ferrisia virgata* (Cockerell), were started at UCB for testing as an alternate host for both native and introduced parasitoids. On one occasion *L. epona* did successfully parasitize striped mealybugs when confined in a small vial. Repeated attempts to culture any of the parasitoid species on striped mealybug failed. Behavioral observations showed that the mealybug's long, glassy spines foul the parasitoids' antennae, and in most cases the parasitoid cannot get close enough for oviposition.

1c.) Evaluate the quality and quantity of parasitoids produced by longtailed mealybug

The second generation *P. angelicus* reared on longtailed mealybug may be somewhat smaller in size than those reared on grape mealybug, which would suggest that longtailed mealybug is not an optimum host for the parasitoid. These differences are currently being quantified.

1d.) Test the citrus mealybug, Planococcus citri, and the citrophilus mealybug, Pseudococcus calceolariae, as potential hosts for a cecidomyid midge

Cultures of an as-yet unidentified cecidomyid mealybug predator are being started in Fall '98. If, as suspected, the species proves to be *Diadiplosis koebelei*, then the citrophilus mealybug should prove to be a satisfactory host. *D. koebelei* was reared and released in southern California in 1928 for use against the citrophilus mealybug. This mealybug has proven exceptionally easy to rear.

2.) Determine the effect of inoculative release of A. notativentris, P. angelicus, and a cecidomyid midge on the population levels of grape and longtailed mealybugs.

2a.) In cage trials, test release of natural enemies (A. notativentris, P. angelicus, and a cecidomyid midge) separately and together as an initial determination of field effectiveness.

Small colonies of grape mealybug have been established on caged grape vines in the UCB greenhouses. These will be used to test parasitoid effectiveness as soon as parasitoid colonies are sufficiently large and free of hyperparasites.

2b.) In on-farm trials, test the effectiveness of an inoculative release of natural enemies.

Farmer collaborators have been identified for potential field trials of *P. angelicus* and/or *A. notativentris* in the 1999 growing season. The releases will take place in pesticide-free fields with a history of serious mealybug infestations, within one month of bud break (usually in early March). These releases are dependent on the continued success of the UCB mealybug and parasitoid colonies.

3.) Release imported natural enemies against the obscure mealybug and determine the effect of natural enemies (*Pseudaphycus flavidulus, Leptomastix epona*, and *Cryptolaemus montrouzieri*) in Central Coast vineyards and *C. montrouzieri* in Carnerros region vineyards.

A review of the literature shows that 11 parasitoids have been introduced in California for biological control of the closely related grape, obscure and longtailed mealybugs, with two showing evidence of establishment (see Attachment C). Eight of these were introduced for control of the longtailed mealybug, three for the obscure mealybug, and one for the grape mealybug . Two of the obscure mealybug parasitoids were imported from Chile recently by K. Daane. *Pseudaphycus flavidulus* (Brethes) was imported and released in 1993 at a large San Luis Obispo vineyard, and successfully overwintered there. Parasitoids were recovered after release during the next year; however, much of the mealybug and parasitoid populations were decimated by late-season sprays of methomyl. Additional *P. flavidulus* were imported in 1997 and releases have continued in 1997-98. Another encyrtid, *Leptomastix epona* (Walker) was imported from Chile in 1997, and has been released in San Luis Obispo and Santa Maria vineyards since late 1997. Establishment of *L. epona* has not been fully assessed, but mummies were found in 1998 in fields that previously had none. Rearing of the two imported parasitoids continues at the UCB lab.

Earlier importations of *C. montrouzieri* have proven unable to overwinter in California's inland valleys. Prof. K. Hagen of UCB imported *C. montrouzieri* from a cooler region of Australia, hoping that this strain would prove more cold-tolerant. The strain was released in the Carnerros region of Sonoma County, which is heavily infested with grape mealybug. While the beetle has been recovered in previous years, none were recovered during the 1998 season. We are continuing to research and release this biotype on a small scale.

The Carnerros region is notable for its lack of parasitism commonly found in Central Valley vineyards. One possibility is that the Carnerros strain of grape mealybug is actually a separate biotype or species, which is an unsuitable host for *A. notativentris* and *P. angelicus*. Tests are now being conducted to ascertain whether these two parasitoids can successfully reproduce on the Carnerros strain of grape mealybug. In preliminary experiments, *P. angelicus* successfully reproduced on the Carnerros strain, and *A. notativentris* aggressively oviposited in third instars.

Attachment A

Personal communications regarding mealybug parasitoid rearing

Walt White, Biotactics Insectaries
John Freeman, Sespe Creek Insectaries
Jim Davis, American Insectaries
Lance Osborne, University of Florida
John Dunley, University of Washington
Dale Meyerdirk, USDA-APHIS
Oxnard Pest Control Association
Wyatt Cone, University of Washington

Attachment B

A preliminary evaluation of rearing media for five species of mealybugs at the UCB lab. Blank cells indicate combinations not tested, + means suitable, - means unsuitable.

Medium	Citrus/ citrophilus	Longtailed	Grape	Obscure
Non-sprouting potatoes	+	- -	- -	-
Sprouting potatoes	++	+	++	++
Acorn squash				- -
Butternut squash			++	++
Kabocha squash		- -	++	
Ice plant			+?	
Pothos ivy		- -		
Dracaena.		+		

Attachment C

Parasitoid species of grape, longtailed and obscure mealybugs that have been recorded or introduced in California (Sources: Clausen 1978, Krombein et al. 1979, and Noyes & Hayat 1994), with comparison of past and recent San Joaquin Valley surveys (Clausen 1924, Daane unpublished data).

Parasitoid Species (Hyperparasitoids not listed)	Mealybug Hosts of Record*	Imported for*	Esta-blished **	Present in 1919 Surveys	Present in 1995 Surveys
Acerophagus notativentris (Girault)	G,O			Y	Y(G)
Acerophagus pallidus Timberlake	G?				
Acroaspidia myrmicoides Compere & Zinna	G?,L	G?,L	E		
Anagyrus fusciventris (Girault)	L	L	N		
Anagyrus clauseni Timberlake	G			Y	
Anagyrus kivuensis Compere	G?, O?	O?	N		
Anagyrus pseudococci (Girault)	G,L,O	L	N (L) E (G?)		
Anagyrus yuccae (Coquillett)	G			Y	
Bothriocraera bicolor Compere & Zinna	G?,L	G?,L	N		
Chrysoplatycerus splendens (Howard)	G,L,O				
Coccidoxenoides (=Pauridia) peregrinus (Timberlake)	G				
Coccophagus gurneyi Compere	G?,L				
Euryhopalus saccharicola (Gahan)	G?,L	L	R		
Encyrtus albicoxa (Ashmead)	L?				
Leptomastidea abnormis (Girault)	L, G				
Leptomastix dactylopii (Girault)	L[1], G[1]				
Leptomastix epona (Walker)	O	O	R		
Pseudaphycus angelicus (Howard)	G,L				Y(G)
Pseudaphycus flavidulus (Brethes)	L,O	O	R	n/a	Y(O)
Pseudaphycus mundus Gahan	L	L	N		
Pseudaleptomastix squammulata Girault	G		Y	Y	
Tetracnemoidea brevicornis (Girault)	L,G?				
Tetracnemoidea peregrina (Compere)	L,G,O	L	P(L)		
Tetracnemoidea sydneyensis (Timberlake)	L	L	P(L)		
Tropidophryne melvillei Compere	L[1]G[1]	G?	N		
Zarhopalus corvinus (Girault)	G		Y	Y	Y(G)
Zarhopalus sheldoni Asmead	G,L[1]				

*Only grape (=G), longtailed (=L) and obscure (=O) mealybug hosts are listed.

**R=released but establishment not yet certain, N=released but not established, E=established with level of control not unknown, P=established with partial control

[1]A species that can attack these grape pests but that are not normally found on these mealybugs.